OBSERVATIONS ON *PLEIONE FORMOSANA* HAYATA[1]

YUNG-REUI CHEN[2]

ABSTRACT

This report is a continuation of my studies on *Pleione formosana* Hayata and deals with the internal structure and differentiation of the roots, pigmentation, variation and differentiation of unfertilized flowers (Chen, 1968).

The primordia of adventitious roots are hidden in the unbroken buds, with numbers ranging from several (3 or 4) to thirty. In the transection of the mature root, there are two to several layers of velamen whose cytoplasm and nucleus disappears from the cell that enclose the cylinder. They are strongly lignified and easily shed. The exodermis takes the place of the function of velamen after the latter dies. Cortical cells are nearly circular and isodiametric. Beneath the endoderm, there are 2 to 3 layers of thick-walled cells surrounding the vascular bundle. The pericycle is absent. The central pith is concealed by radiate vascular tissue. In the longisection of the root, all the tissues originate from one group of initials, *i.e.* root cap, velamen, cortex and vascular cylinder are all differentiated from one group of cells.

The flowers of *Pleione* are dioecious and epigynous. They contain a 3-carpeled ovary and an anther with floral appendages. During the blooming period, cross-pollination occurs. After pollination both the pollen and the nucellus ripen. In the apical meristem a second flower remains undeveloped. The natural occurrence of a second and a third flower on one scape is an evidence of its tendency to form a spike.

By analyzing the pigments in purple flowers it is found that there are four pigment groups, *i.e.*, anthocyanins, flavones, flavonols and aurones. But in white flowers only flavones and aurones are found. The result on the paper chromatogram shows that six kinds of anthocyanins are found in flowers and these are different from those in pseudobulbs. The maximum absorption of the anthocyanin extract is at 520 mμ.

The abscission of the leaf is almost the same as that of the pedicel. The nuclei disappear and cell walls do not stain with safranin in the cells beyond the abscission layer, but the cells connected with the plant body are lignified and stain red with safranin.

INTRODUCTION

Pleione formosana Hayata is a native orchid of Taiwan. It grows on alpine rocks at 1,000 m to 2,500 m above sea level. In the spring, the annual pseudobulbs

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usually produce a new leaf. Sometimes 2 or 3 leaves may develop at the tip of a pseudobulb. When tbe blooming period is near, the flowers, 1 or 2, arise from the slit between the sheath of leaf. Tbey are large, bright and colorful.

This orchid was first collected by U. Mori (1909) and named by B. Hayata (1911). After which it was studied by several botanists including: Fukuyama (1932, 1934), Hayata (1914, 1919), Kudo (1934), Rolfe (1917) and Schlecbter (1919). In the author's master's thesis (1968) and Chiang & Chen (1968), be discussed this orchid dealing with its nomenclature, external features, internal structure, flowering time and tbe pigmentation of the pseudobulb. He recognized *Pleione formosana* Hayata as the only native species of *Pleione* in Taiwan. In tbe present study, the author is continuing his work on the structure and development of the flower before fertilization, the pigmentation of the flower and the abscission of tbe leaf.

MATERIALS AND METHODS

All plant materials were either collected from the green bouse of the Botany Department of the National Taiwan University or from tbe field. For anatomical studies plant materials were fixed in FAA, infiltration with t-butanol series and embedded in paraffin. Serial sections were made 8 μ in thickness, tben dehydrated witb an alcobol series and stained witb safranin O and fast green (Johansen, 1940). For studying the pigments of the flowers, 10 g floral parts were extracted with 95% methanol (40 ml) for 2 days and filtered. Tbe filtered extract was concentrated to 3–4 ml on a water batb. Tbe pigment extracts and fresh tissues of flowers were tested by the previously employed method (Chen, 1968). Anthocyanins were separated by paper chromatograpby (Endo, 1954). Tbe solvent used was the upper layer of n-butanol–36% HCl-water (5:1:4). Tbe development was carried out in a glass cylinder in the dark by the ascending method. Spectronic-20 was used for determinating the absorption spectrum of anthocyanins (Arditti and Dunn, 1969).

RESULTS

1. Root anatomy.

Tbe internal structure of the root from mature plants of *Pleione* is shown on Plates 1 and 2. At the region of root tip, all the tissues are derived from one

Plate 1. Diagrammatie illustrationa of the apical meristem and derivatives in the root.

A, median longisection of a diagram of the roots showing the appearance and maturation of different tissuea at various levels. B, transection of root at 80 μ from the root tip. C, tran aection of root at 860μ from the tip showing the matnration of sieve elements. D, transection of root at 2680μ from the root tip, showing that the maturation of the xylem occurs long after that of the phloem. E, transection of the mature root showing that the outermoat layers of ceils in the vascular bundle are atrongly lignified.

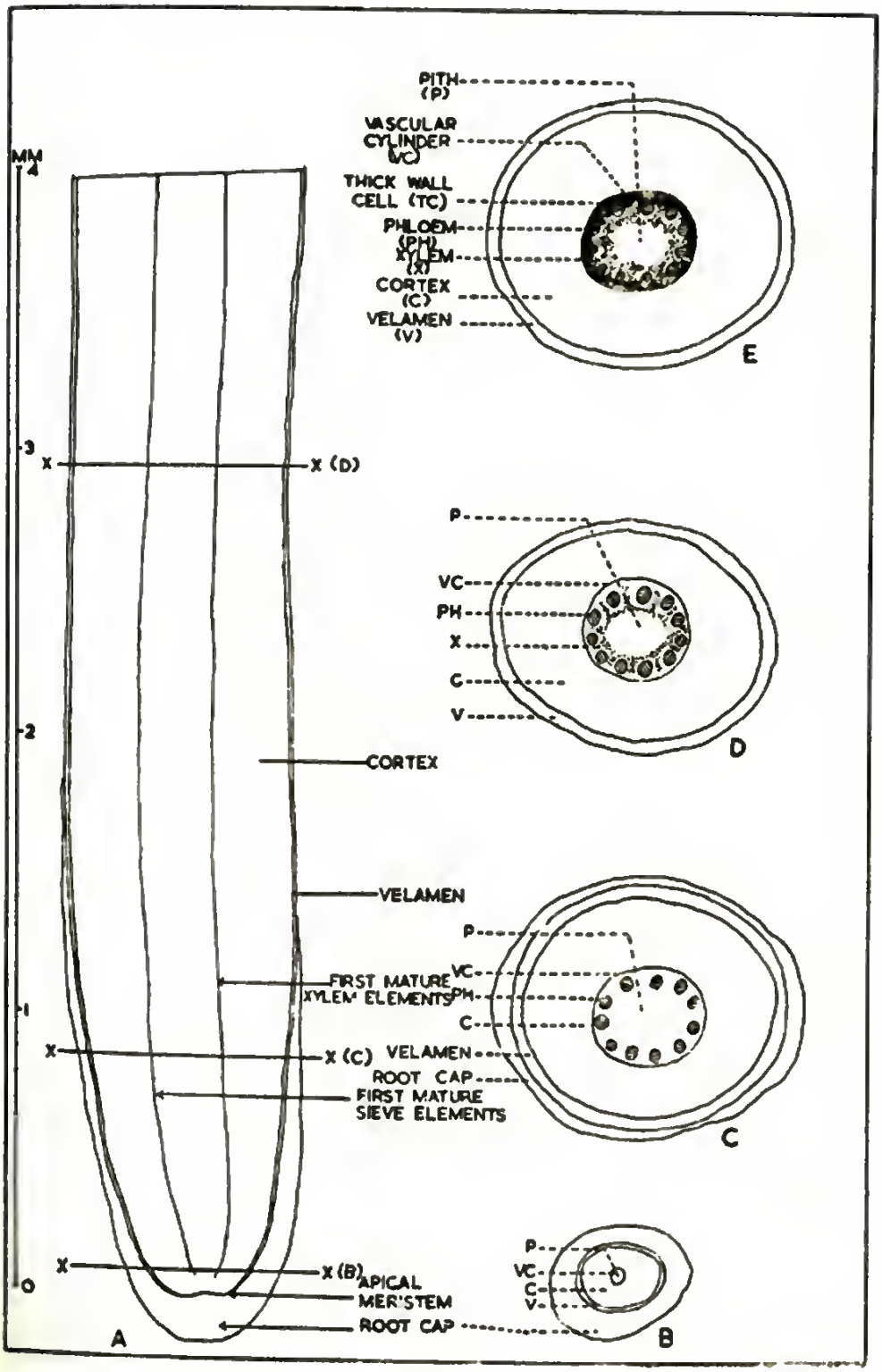
MM
4
3
2
1
0
PITH (P)
VASCULAR CYLINDER (VC)
THICK WALL CELL (TC)
PHLOEM (PH)
XYLEM (X)
CORTEX (C)
VELAMEN (V)
E
X
X (D)
P
VC
PH
X
C
V
D
CORTEX
VELAMEN
FIRST MATURE XYLEM ELEMENTS
P
VC
PH
C
X
X (C)
VELAMEN
ROOT CAP
FIRST MATURE SIEVE ELEMENTS
C
X
X (B)
APICAL MERISTEM
ROOT CAP
A
P
VC
C
V
B

Plate 1

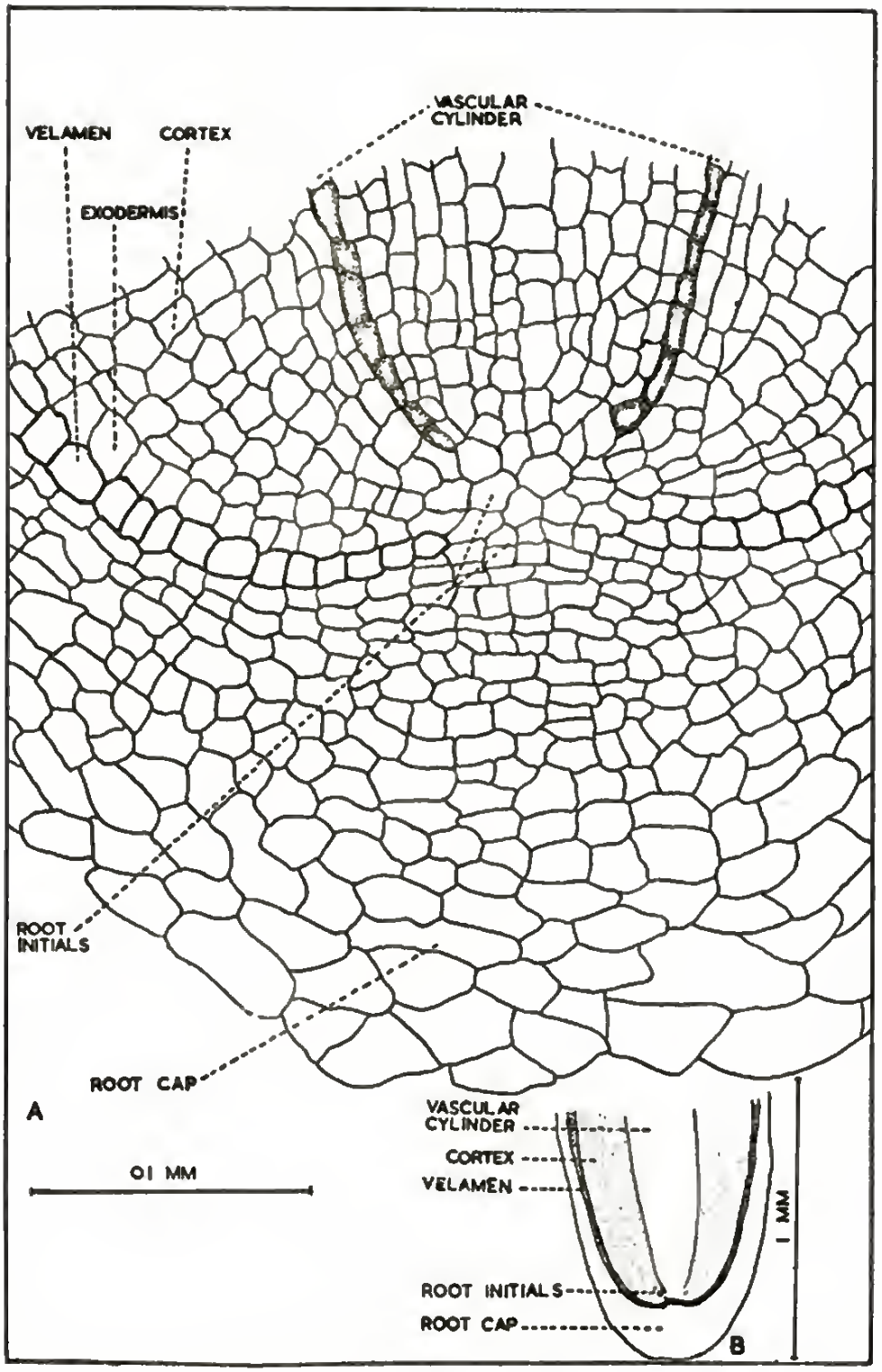
VELAMEN
CORTEX
EXODERMIS
VASCULAR CYLINDER
ROOT INITIALS
ROOT CAP
A
01 MM
VASCULAR CYLINDER
CORTEX
VELAMEN
ROOT INITIALS
ROOT CAP
1 MM
B

Plate 2

initial. That is, the root cap, velamen, cortex and vascular cylinder are without clear boundaries. There are, at least, 6 to 10 cells formed from the initial, and they are similar in shape and size.

Root cap.

As shown in Plates 1 and 2, the root cap is like a hat covering the apical meristem decreasing in thickness until it reaches the maturation zone of protective tissues, *i. e.*, velamen and exodermis. The innermost layer of the root cap is different from the velamen in that it is more vacuolated (Plates 9A and 9B). In the first 5 or 6 layers centrifugal to the initial, periclinal divisions predominate and cells are arranged regularly. In the next 6 to 10 layers anticlinal divisions are predominate, and the cells are concentric. In general, the cells of the root cap vary in size ranging from 10 to 50 μ. The older cells of the root cap are highly vacuolated and finally break up.

Velamen (Epidermis).

The velamen is a multiseriate epidermis (usually 2 or 4 layers thick) which is uniseriate and invaginated at the tip of root (Plates 2A, 3A and 9C). The anticlinal divisions occur first and subsequently periclinal divisions occur (Plate 3B). In transverse section of the root (Plate 9C), there are 2 layers of cells arranged in a radial pattern. The velamen cells are strongly lignified and the cytoplasm is absent at maturity. The thickness of the radial wall is much greater than that of the tangential walls. Root hairs are white and are derived from the outermost layer of the velamen (Plate 3C). When the roots are exposed to the light they turn green if they are moist, but turn white when they are dry.

Cortex.

The cortex is compactly arranged beneath the velamen. In the mature root, the cortex can be divided into three regions: the exodermis, the cortex proper and endodermis. The exodermis, which is the outermost layer of the cortex, has larger cells and are vacuolated so that they can be easily distinguished from the velamen. At maturity the exodermal walls are thick and lignified (Plate 9C), and their elongation is parallel to the axis of root. Between the exodermal cells, occasionally, thin-walled passage cells are present for the transportation of liquids to the velamen. The cortex proper consists of isodiametrical parenchymatous cells. Intercellular spaces are universally present in the cortex except in the outermost layer (Plate 9C). Idioblasts, with raphides, are first observed about 200 μ above the rim of the

Plate 2. Apical meristem and derivative region in root.

A, longitudinal section of the root tip of a mature plant. The heavy black line delimits the velamen from the root cap, and the dotted shaded cells delimits the vascular cylinder from the cortex. B, diagrammatic longitudinal section of root showing the root initials and various zones.

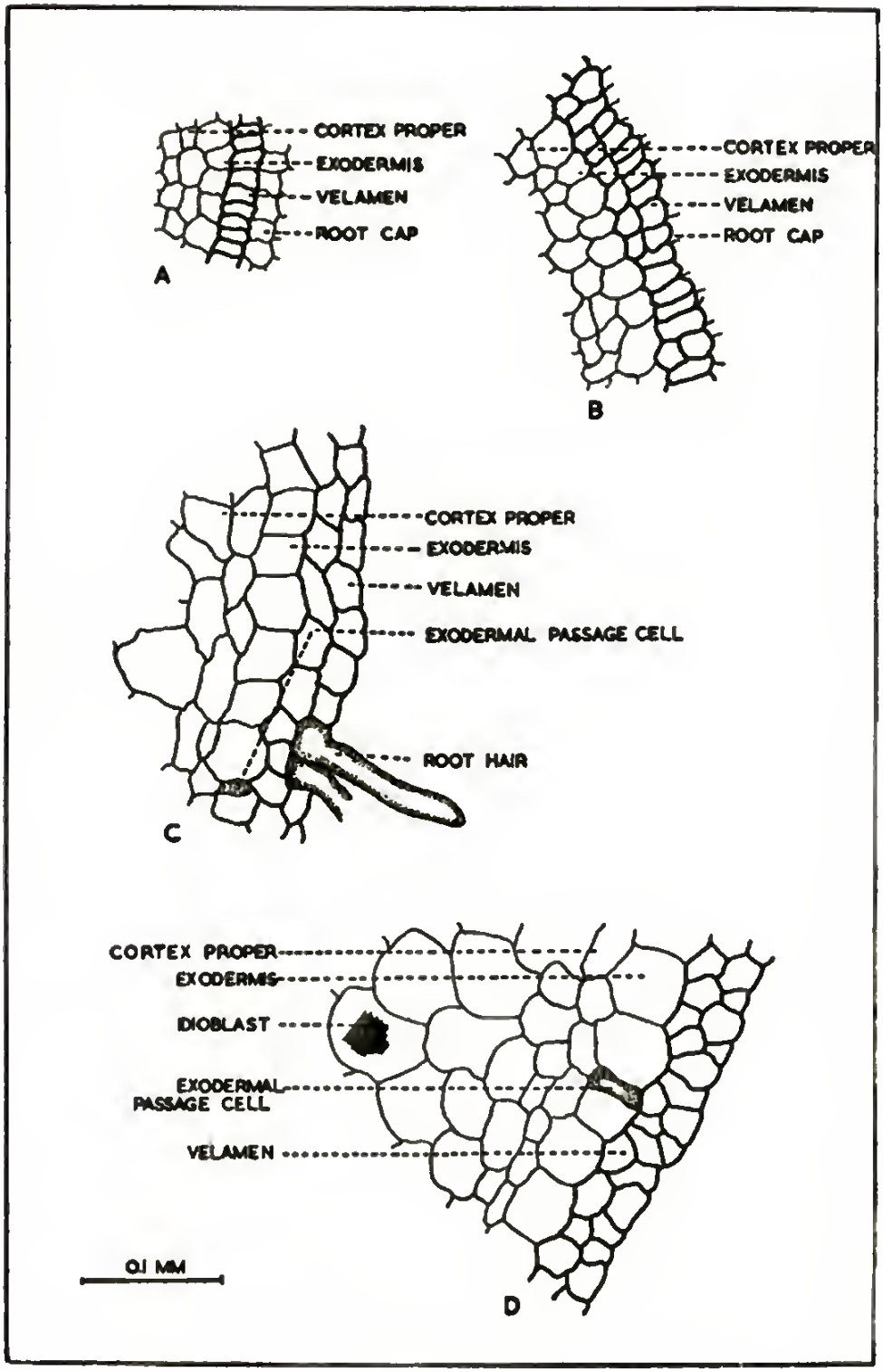
CORTEX PROPER
EXODERMIS
VELAMEN
ROOT CAP
A
CORTEX PROPER
EXODERMIS
VELAMEN
ROOT CAP
B
CORTEX PROPER
EXODERMIS
VELAMEN
EXODERMAL PASSAGE CELL
ROOT HAIR
C
CORTEX PROPER
EXODERMIS
DIOBLAST
EXODERMAL PASSAGE CELL
VELAMEN
0.1 MM
D

Plate 3

tip and are much larger than the surrounding cells. The endodermis, surrounds the radial siphonostele with the alternating segments of thin-and thick-walled cells. The thick-walled cells are used as a dam for preventing the loss of water and the thin-walled cells serve as passage cells for the translocation of food and minerals.

Vascular cylinder.

The vascular cylinder, is composed by three tissues, *i.e.*, xylem, phloem and pith (Plate 9C). Unlike the perennial orchids, the pericycle is lacking. Lateral roots and secondary growth, therefore, does not take place. The vascular tissues are arranged compactly in a radial alternating form. The central part of the vascular bundles is pith. As shown in Plate 2, the cells in the pith first appear during the development of vascular tissue. They are elongated and parenchymatous (Plates 2A, 9A, 9B and 9C). Intercellular spaces are present in the pith (Plate 9C). Sieve elements first appear and mature respectively at 400 μ and 700 μ above the rim of root tip. In the mature root, phloem tissue is located opposite the thick-walled cells which are 2 to 3 layers in thickness (Plate 9C). The first mature protoxylem is found at 1100 μ above the tip of root. The immature metaxylem first appears at 700 μ but it is mature at 1440 μ above the root tip.

Root primordium.

As can he seen in Plates 9D and 10A, several root primordia are present in the unbroken buds. The origin of these roots is from opposite vascular bundles, which are distrihuted in the hud. These root primordia are all well differentiated. They consist of root cap, epidermis, cortex and vascular cylinder.

2. Flower.

The flowers of *Pleione* are dioecious and epigynous (Plate 4), and contain a 3-carpeled ovary and a column with the floral appendages, *i.e.*, sepals, petals and lip. The floral appendages are arranged counterclockwise in two whorls. A terminal anther with 4-pollen sacs is located on the tip of column (Plate 4A). In each pollen sac, the pollen grains are aggregated and called a pollinium (Plate 6A). Beneath the anther there is a stigma secreting nectar for attracting insects. The inferior ovary has 3 parietal ridges. In the transection of the flower (Plate 4), an apical meristem with a second flower is seen, remaining undeveloped beside the growing flower. The natural occurrence of a second and a third flower on one scape is an evidence of its tendency to form a spike.

Plate 3. Transverse section showing the development of the velamen and exodermis.

A, section just above the root tip. **B,** section at 300 μ above the rim of tip ahowing the 2-seriate velamen. **C,** section at 2000 μ from the rim of tip ahowing that root hairs are derived from the velamen and that passage cell ia located between two exodermal cells. **D,** section at 5 cm above the rim of tip ahowing an idioplast with its raphida in the cortical tissue.

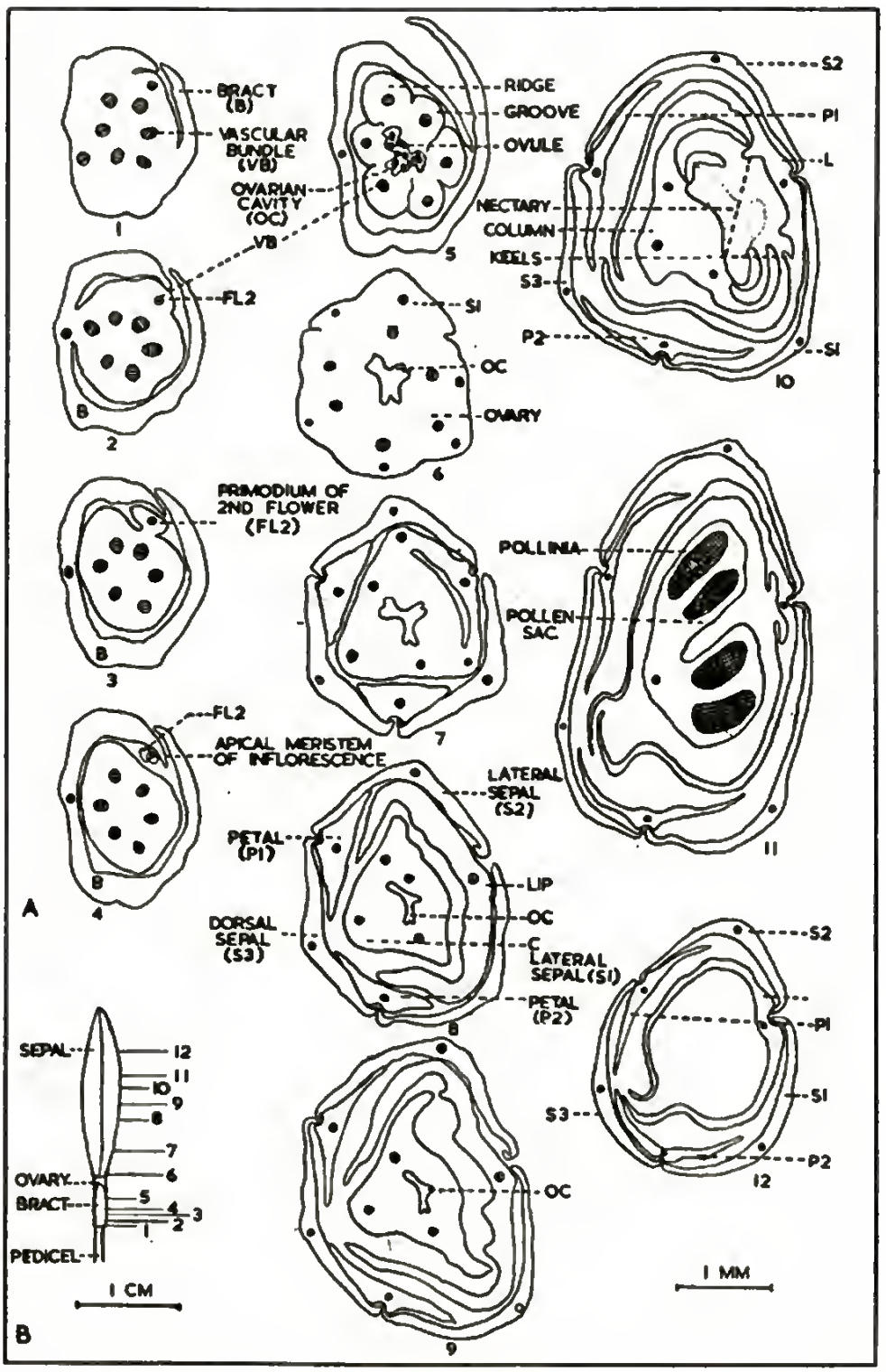
BRACT (B)
VASCULAR BUNDLE (VB)
OVARIAN CAVITY (OC)
VB
FL2
B
1
2
RIDGE
GROOVE
OVULE
5
S2
PI
L
NECTARY
COLUMN
KEELS
S3
P2
SI
10
SI
OC
OVARY
6
PRIMODIUM OF 2ND FLOWER (FL2)
3
POLLINIA
POLLEN SAC
7
11
FL2
APICAL MERISTEM OF INFLORESCENCE
4
LATERAL SEPAL (S2)
PETAL (P1)
LIP
OC
C
DORSAL SEPAL (S3)
LATERAL SEPAL (S1)
PETAL (P2)
8
A
S2
PI
SI
S3
P2
12
SEPAL
12
11
10
9
8
7
OVARY
6
BRACT
5
4
3
2
1
PEDICEL
1 CM
OC
9
1 MM
B

Plate 4

Mature pollen grains contain a generative nucleus and a tube nucleus (Plate 6C). The former is characteristically smaller, lens-shape and darkly stained while the latter is larger and lightly stained.

A transverse section of the ovary fixed before pollination (Plate 5A and 10C) shows there are three parietal ridges on which branched protuberances develope. The tips of the protuberances arise as an axial row of eight nucellar cells surrounded by the nucellar epidermis which is arranged in two rows. The terminal nucellar cell is called the archesporial cell which gives rise to the megaspore.

3. Identification of pigments in flowers.

Three kinds of flowers, *i.e.*, purplish, pinkish-white and white were used for identifying the pigments present in the flower. The results are shown in Table 1.

Table 1. The relative amounts of water-soluble pigments in various flowers of *Pleione formosana* Hayata*

Color of flower	Anthocyanin	Flavone	Flavonol	Aurone
Purple	++	+	++	±
Pinkish-white	+	+	+	++
White	−	±	−	+

* ++ denotes the presence of pigment in high concentration. + denotes the presence of pigment in low concentration. ± denotes the presence of a pigment in trace amounts. − denotes the absence of pigment.

The purplish and pinkish white flowers contain 4 kinds of pigments, *i.e.*, anthocyanins, flavones, flavonols and aurones, but these are present in different amounts. On the other hand, the white flowers contain only aurones and a small amount of flavones.

Table 2. Characteristics of anthocyanins in the sepals and petals of *Pleione formosana* Hayata.

Spot number	Color		Rf value (BAW)*	Relative amounts
	Visible light	UV light		
a	Yellow	Brownish-yellow	0.57	6
b	Orange-red	Purple	0.30	4
c	Purplish-red	Violet	0.21	3
d	Purplish-red	Violet	0.17	1
e	Purplish-red	Violet	0.12	2
f	Pink	Purplish-red	0.03	5

* BAW: *n*-butanol-36% HCl-water (5:1:4).

Plate 4. Structure of a growing flower before blooming.

A, transections of a flower before opening at different levels. 1, 2, 3, 4,...., 11 and 12 are respective sections through a flower which are shown in Fig. B.

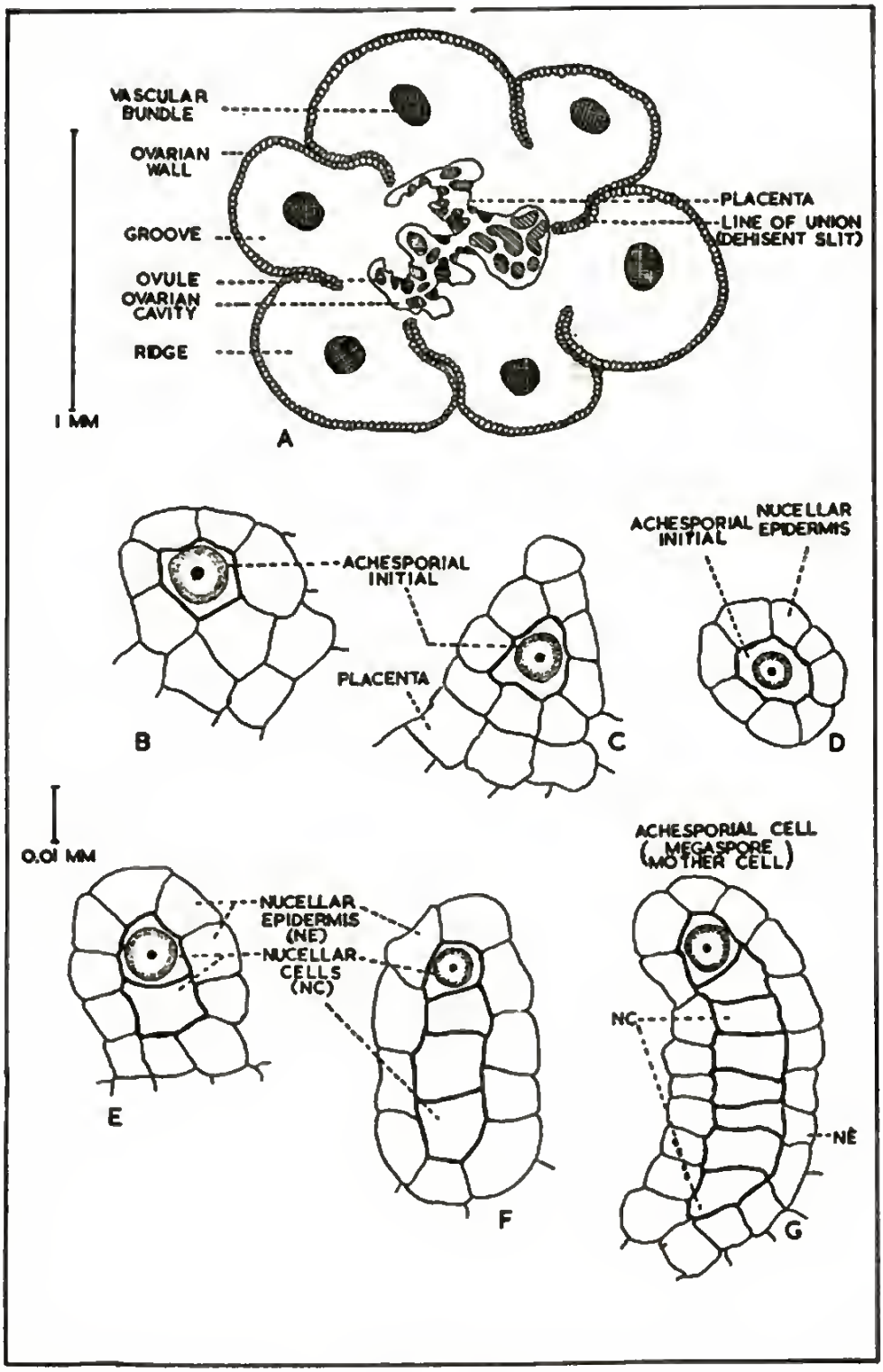
VASCULAR BUNDLE
OVARIAN WALL
GROOVE
OVULE
OVARIAN CAVITY
RIDGE
1 MM
A
PLACENTA
LINE OF UNION (DEHISENT SLIT)
ACHESPORIAL INITIAL
PLACENTA
B
C
ACHESPORIAL INITIAL
NUCELLAR EPIDERMIS
D
0.01 MM
NUCELLAR EPIDERMIS (NE)
NUCELLAR CELLS (NC)
E
F
ACHESPORIAL CELL (MEGASPORE MOTHER CELL)
NC
NE
G

Plate 5

Paper chromatography indicates that the anthocyanins in the flower are different from those of the pseudohulh. Six spots were ohserved (Tahle 2). The maximum ahsorption spectrum of the anthocyanin extract is at 520 mμ.

4. Abscission of leaf.

The ahscission of the leaf is almost the same as that of pedicel (Eames and MacDaniels, 1947). Before the leaf falls (or before the flower falls), an ahscission layer is present at the base of the petiole (or pedicel). After the leaf falls, the cell walls below the ahscission layer become lignified, and are stained with red by safrnin O (Plate 10D).

5. Integration of vascular tissues.

As shown in Plate 7 and Tahle 3, the vascular hundles are distrihuted evenly except at the region from 0 μ to 200 μ ahove the rim of root tip. The phloem serves for translocation of food from the synthesizing and storage organs to the utilizing organs, *i.e.*, root, flower and fruit. It is clear that the roots, the ahsorbing organs, need strengthening tissues for conducting water upward and the phloem/xylem ratio is low (0.1). The reproductive organs, on the other hand, need greater amount of food for forming now tissues and the walls of the reproductive organs lack stoma for transpiration, thus the phloem/xylem ratio is very high in them (2.3 to 4.1). That the phloem/xylem ratio of the leaf is low (0.42) is due to its great amount of evaporation. The pseudohulh functions as a stem in translocation and as a storage organ, and its phloem/xylem raito is near to 1. The ahove facts indicate that the vascular tissue functions as a whole.

Tahle 3. The area ratio of phloem and xylem in vascular bundles of various organs.

	Root		Pseudo-hulh (Stem)	Leaf		Flower		
	Tip	Mature region		Petiole	Blade	Pedicel	Ovary	Column
Relative area* of xylem	0	56.50	10.00	8.25	8.25	2.90	2.40	2.20
Relative area* of phloem	3	7.00	8.10	3.50	3.50	5.50	5.50	9.00
Phloem/xylem		0.12	0.81	0.42	0.42	1.90	2.29	4.09

* Both the relative areas of phloem and xylem are measured hy the weighing method, comparing the weights of tracing paper which represent the areas of phloem and xylem after enlargement.

Plate 5. Stages in the development of the archesporial cell.

A, median transverse section of the ovary at the time of pollen matnration showing the placenta ridge, and line of union (dehiscent slit). B, archesporial initial cell on the placenta ridge. C, late atage of archeaporial initial cell. D, transverse section of C. E and F, 2-cella and 4-cella atages ahowing the filamentous row of nucellar epidermis. G, finai atage of archesporial cell develapment.

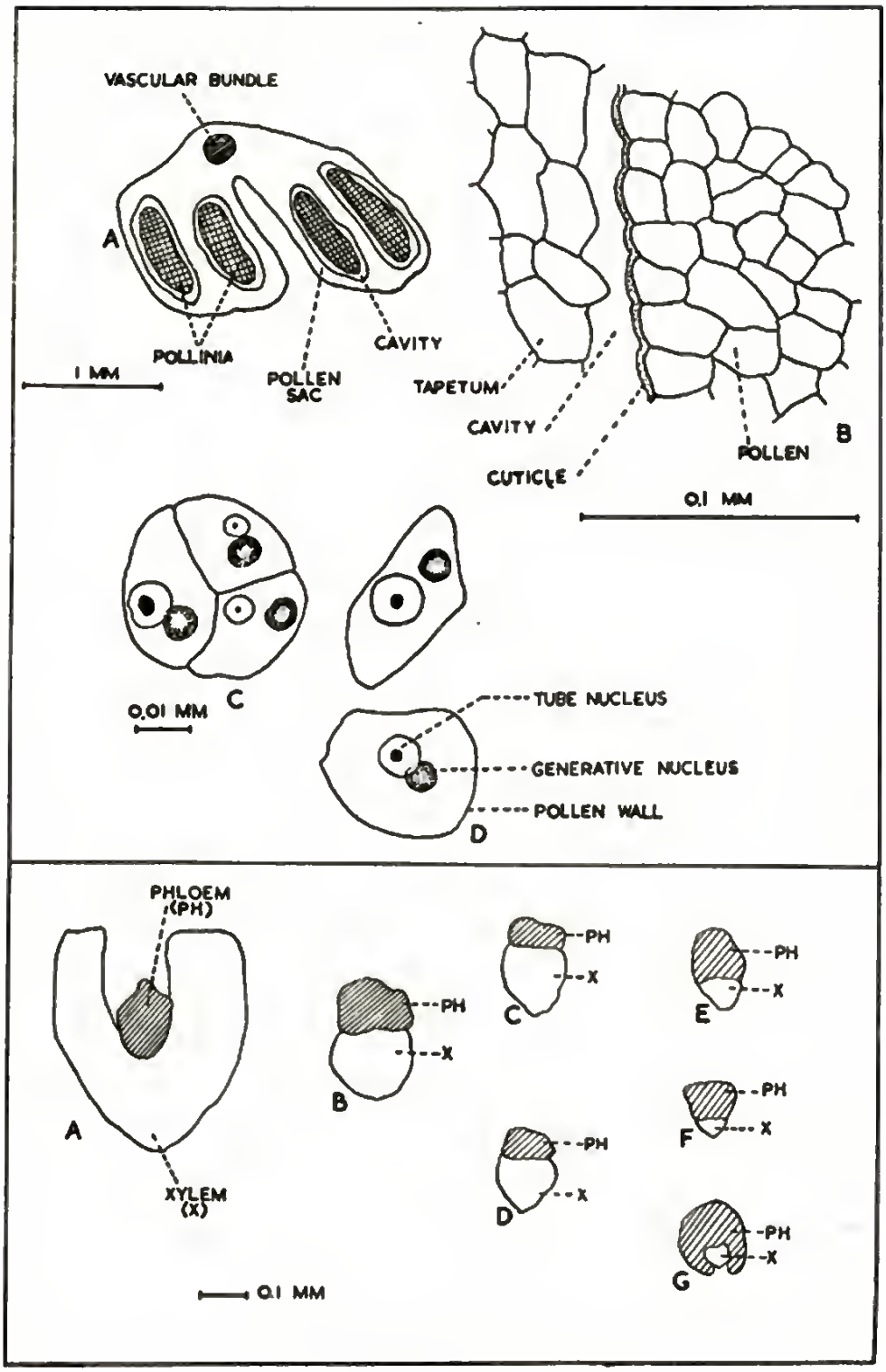
VASCULAR BUNDLE
A
POLLINIA
POLLEN SAC
CAVITY
1 MM
TAPETUM
CAVITY
CUTICLE
POLLEN
B
0.1 MM
0.01 MM
C
TUBE NUCLEUS
GENERATIVE NUCLEUS
POLLEN WALL
D
PHLOEM (PH)
A
XYLEM (X)
PH
X
B
C
D
E
F
G
0.1 MM

Plate 6 and 7

DISCUSSION

In botanical literature the velamen is usually treated as an organ for the absorption of water (Haberlandt, 1914; Jeffrey, 1914, cited in "The Orchids", Withner ed. 1959), but physiological studies indicate that the velamen is a tissue for mechanical protection and for the reduction in loss of water from the cortex (Dycus and Knudson, 1957). In this study it is found that both the exodermis and velamen are strongly lignified except at the connecting region of exodermal passage cells and the tangential thin-walled cells of the velamen. The passage is used as a course for water penetration. This fact indicates that the velamen may prevent water loss and protect the inner layers from mechanical injure.

The time for the development of male and female gemetes is different. The pollen grains are mature before the differentiation of megaspore mother cell. After the pollen grain has fallen on the stigma, the development of archesporial cells is accelerated, hence the development of the ovule is induced by pollination.

In the transection of a flower before blooming, it is found that there is an apical bud and a dormant second flower bud located near the base of the first flower. In the field, the larger pseudobulbs have more chances of producing 2 or 3 flowers on a scape. All of the above facts indicate that it is possible to produce up to four flowers on one scape and thus the inflorescence of *Pleione* will become a spike when grown under good nutrient conditions.

In the abscission zones of both the leaf and pedicel, the cells are arranged regularly. The distal part beyond the abscission layer dies off. The formation of the abscission layer may be due to the action of pectin methyl esterase which digests the pectic compounds (Osborne, 1958). Heavy rain favors the accumulation of water in the envelop of the sheaths and hastens the hydrolysis of pectic compounds and accelerates leaf fall. Incessant raining may be the cause for *Pleione* plants not blooming in 1969.

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Plate 6. The development of pollen.

A, median transection of an anther with 2 lobes and 4 pollen sacs in which the pollen is aggregated into masses. B, partial enlargement of A showing the outer layers of pollinium. C, tetrahedral tetrad. E, transection of a pollen grain showing the generative and tube nucleus.

Plate 7. Diagrammatic illustration of vascular bundles in various organs.

A, in root. B, in pseudobulb (stem). C, in petiole. D, in blade. E, in pedicel. F, in ovary. G, in column.

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Plate 8. The absorption spectrum of anthocyanin extracts from the sepals and petals of *Pleione*.

The anthocyanin was extracted with 1% HCl-MeOH. The dotted line from the peak to the abscissa indicates the wave-length at maximum absorption.

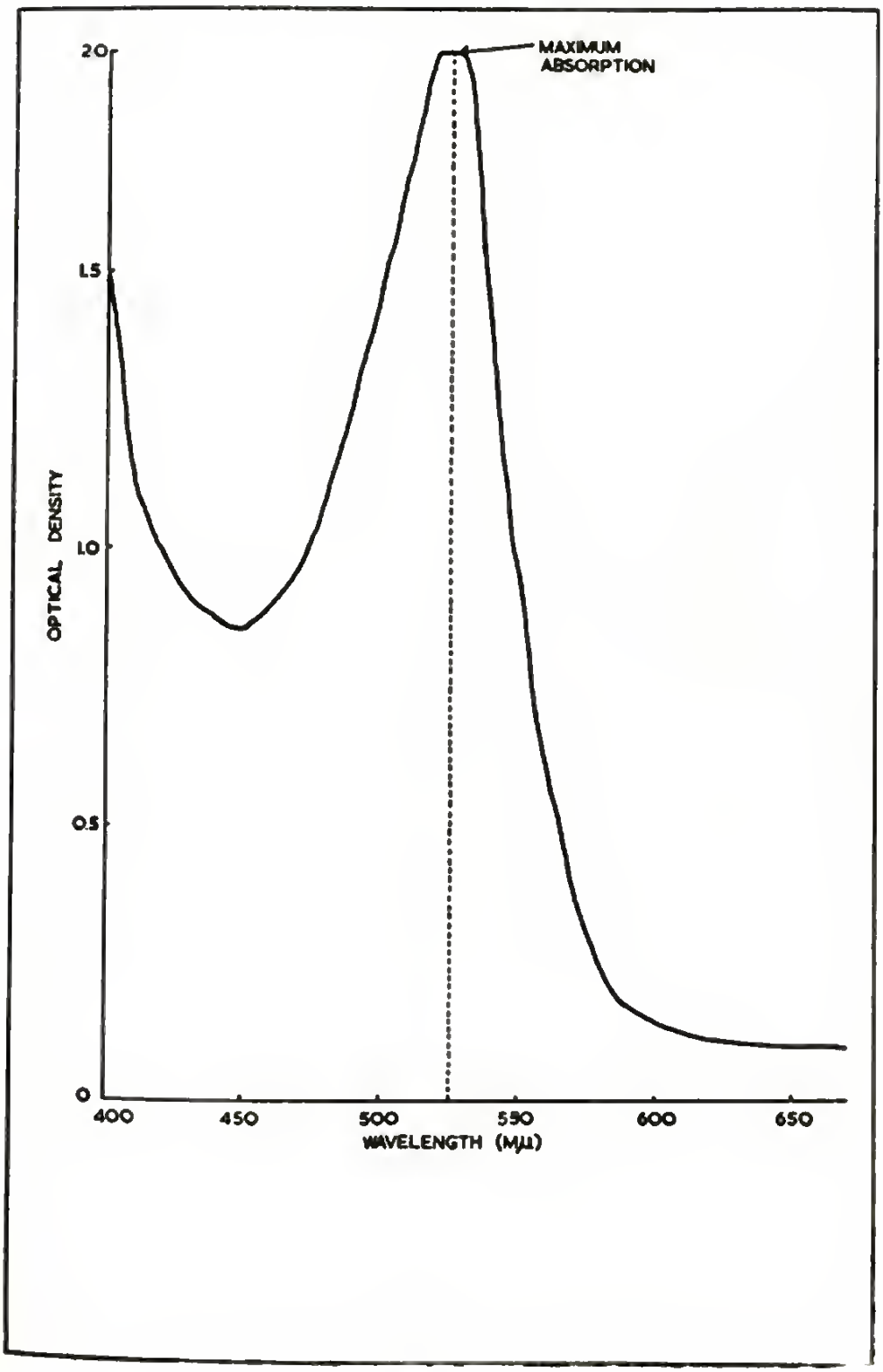
MAXIMUM
ABSORPTION
OPTICAL DENSITY
2.0
1.5
1.0
0.5
0
400
450
500
550
600
650
WAVELENGTH (MμL)

Plate 8

Plate 9. Root anatomy.

A, median longitudinal section of a root showing the various tissues in the root, ×130. B, partial enlargement of A showing the root initial in the apical portion of root, ×150. C, median transverse section of mature root showing the clear boundary of various tissue, ×130.

D, three root primordia in an unbroken bud, ×115.

Key to labelling: C, cortex; D, dermal tissue; En, endodermis; Ex, exodermis; Pi, pith; Rc, root cap; Ri, root initial; Rp, root primordia; THC, thick-walled cell; V, velamen; Ve, vascular cylinder; Vs, vascular bundle; X, xylem.

Plate 10. Miscellaneous organs.

A, root primordium in an unbroken bud showing the apical meristem and root cap in full differentiation before breaking the outer tissues of the bud, ×115. B, various stage of archesporial cells on the placenta, ×488. C, pollen in pollinium showing the tetrahedral microspores, ×518. D, abscission layer of leaf, ×130.

Key to labelling; Ab, abscission; Ac, archesporial cell; C, cortex D, dermal tissue; Gn, generative nucleus; Ne, nucellar epidermis; Oc, ovarian cavity; Pl, placenta; Te, tetrad; Tn, tube nucleus; Ve, vascular cylinder; Vs, vascular bundle.

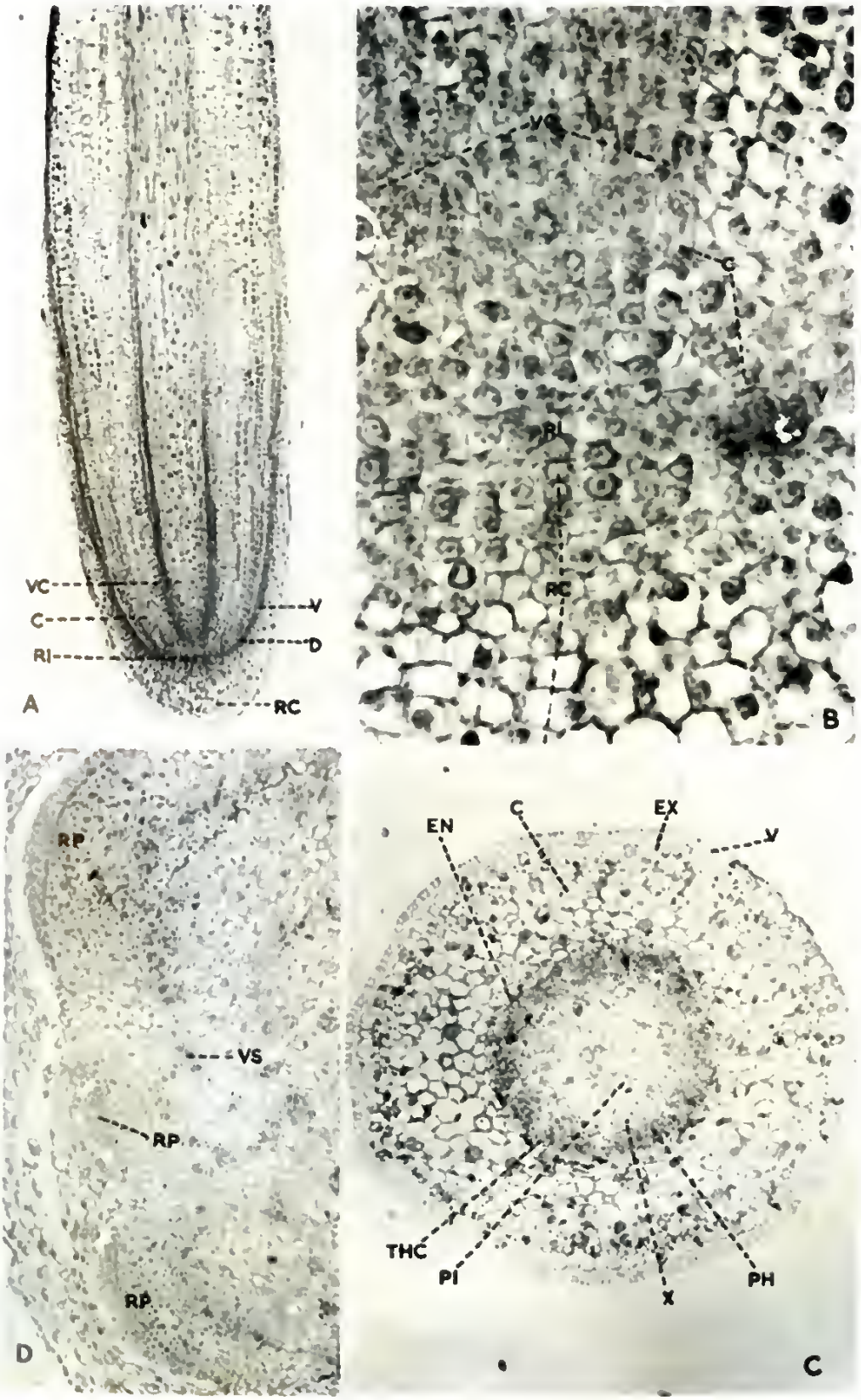

Plate 9

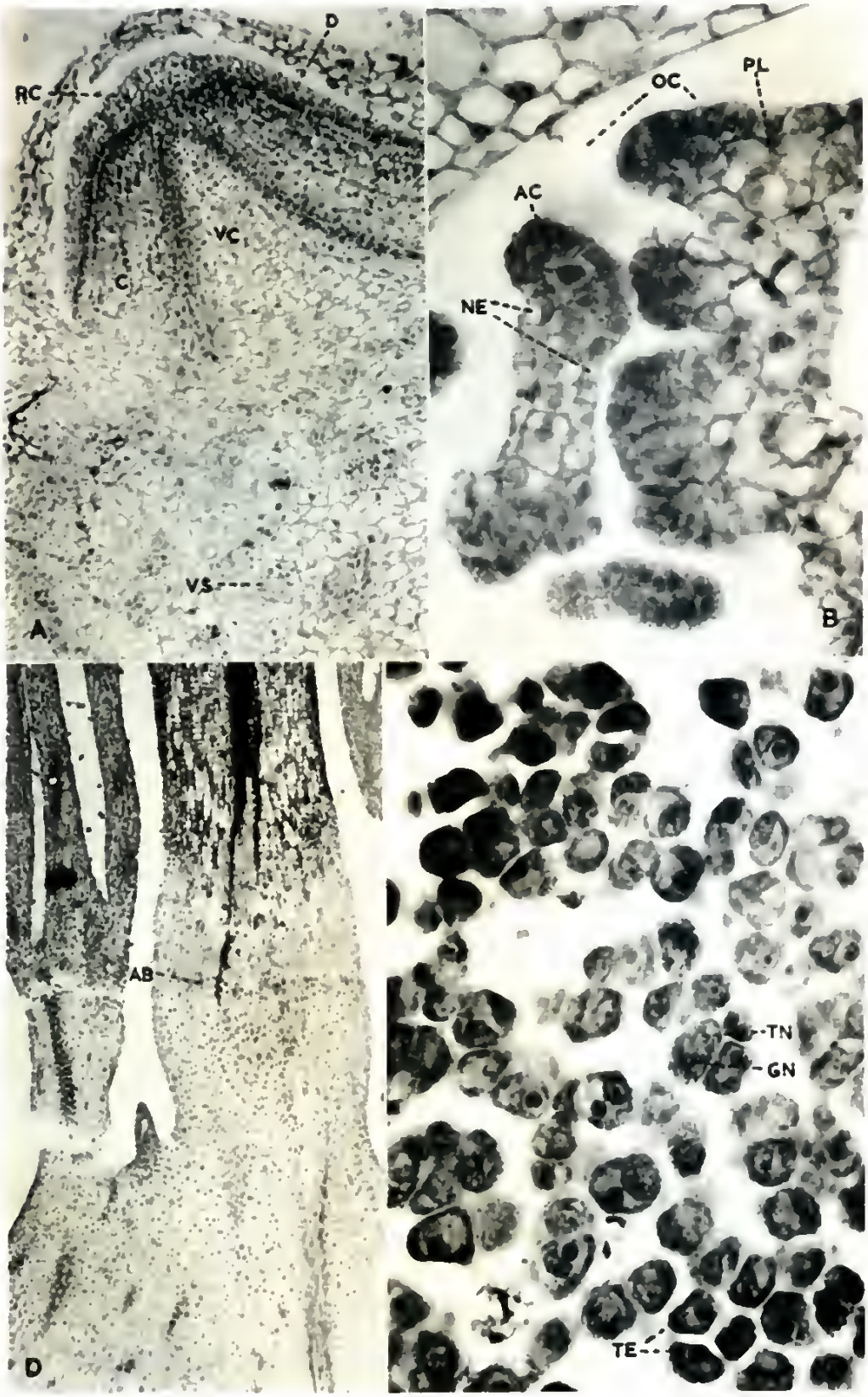

Plate 10